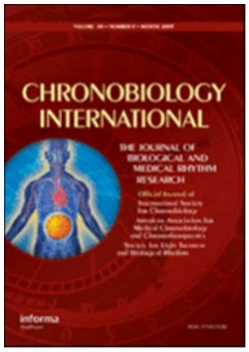

Wrist actimetry circadian rhythm as a robust predictor of colorectal cancer patients survival

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Wrist actimetry circadian rhythm as a robust predictor of colorectal cancer patients survival

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Running title

Rest-activity rhythm as a predictor of survival

Key words

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ABSTRACT

The disruption of the Circadian Timing System (CTS), which rhythmically controls cellular metabolism and proliferation, accelerated experimental cancer progression. A measure of CTS function in cancer patients could thus provide novel prediction information for outcomes, and help identify novel specific therapies. The rest-activity circadian rhythm is a reliable and non invasive CTS biomarker, which was monitored using a wrist watch accelerometer for 2 days in 436 patients with metastatic colorectal cancer. The relative percentage of activity in-bed versus out-of-bed (I<O) constituted the tested CTS measure, whose prognostic value for overall survival (OS) and progression-free survival (PFS) was determined in a pooled analysis of three patient cohorts with different treatment exposures. Median OS was 21.6 months [17.8-25.5] for patients with I<O above the median value of 97.5% as compared to 11.9 months [10.4-13.3] for those with a lower I<O (Logrank $p<0.001$). Multivariate analyses retained continuous I<O as a joint predictor of both OS and PFS, with respective Hazard Ratios (HR) of 0.954 ($p<0.001$) and 0.970 ($p<0.001$) for each 1% increase in I<O. HRs had similar values in all the patient subgroups tested. The circadian physiology biomarker I<O constitutes a robust and independent quantitative predictor of cancer patient outcomes, that can be easily and cost-effectively measured during daily living. Interventional studies involving 24-h schedules of clock-targeted drugs, light intensity, exercise and/or meals are needed for testing the relevance of circadian synchronization for the survival of patients with disrupted rhythms.

INTRODUCTION

Circadian rhythms are potential biomarkers related to health and diseases. These rhythms are generated at single cell level by a molecular clock involving at least 15 specific genes (Dibner et al., 2010). The cellular clocks are coordinated by the suprachiasmatic nuclei (SCN) in the hypothalamus, a pacemaker that further adjusts the circadian rhythms to the environmental day-night cycles (Dibner et al., 2010). Experimental data further show that the network of molecular clocks that constitute the Circadian Timing System (CTS) is a control point for tumor progression. Thus cancer progression was accelerated two- to three-fold in tumor-bearing mice whose rest-activity rhythm was suppressed as a result of SCN ablation or chronic 'jet lag' (Fu et al., 2002; Filipski et al., 2002). Conversely CTS amplification through programmed daily meal timing halved experimental cancer growth in mice (Wu et al., 2004, Li et al., 2010). Here we investigated the clinical relevance of such preclinical findings in a large sample of cancer patients, using non invasive technology.

The rest-activity rhythm constitutes a clinically relevant circadian biomarker, since it is amenable to non-invasive monitoring with a wrist watch accelerometer (Mormont et al., 2000). The quest for circadian biomarkers of human health is further supported by the demonstration of an increased risk of cancer in shift workers exposed to occupational schedules that disrupt circadian rhythms (IARC-Working-Group, 2010). The human CTS is synchronized by environmental light-dark alternation, as well as by socio-professional routine and meal timing, whose iterative and sustained alterations disrupt the CTS (Levi et al., 2010). Such disruption has been documented using several parameters derived from wrist actimetry monitoring (Mormont et al., 2000; Ancoli-Israel et al., 2003; Berger et al.,2008). Several such devices have been approved for human use, yet their specific technical design and the time series analyses methods that are used can influence the parameter values that are obtained. Using the Minimotion logger actimeter®, we first revealed the clinical relevance of the

dichotomy index I<O, a measure of the percentage of in-bed activity counts that are less than the median of out-of-bed counts (Mormont et al., 2000; Minors et al., 1996). Thus, in two previous cohort studies, I<O provided consistent prognostic information on the survival of patients with metastatic colorectal cancer (Mormont et al., 2000; Innominato et al., 2009a). Cohort I was a single institution study that included 192 consecutive patients mostly receiving chronomodulated 5-Fluorouracil-Leucovorin-Oxaliplatin chemotherapy after failure of a first treatment protocol (Mormont et al., 2000). Cohort II consisted of 130 chemo-naïve patients receiving chronomodulated or conventional delivery of the same 3-drug protocol within a randomized international Phase III trial (Innominato et al., 2009a). Such easily measurable circadian biomarkers that predict patient survival outcome have the potential for improving cancer care through the assessment of prognosis, the prediction of response to specific treatments, as well as the identification of novel circadian therapeutics (Buyse et al., 2010).

Here, we first aimed at confirming the prognostic value of I<O as a circadian biomarker within a third cohort of 142 patients with metastatic colorectal cancer. These patients then received salvage multidrug chronotherapy as part of their routine oncology management after failure of chemotherapy for a majority of them. We then determined the prognostic value of I<O in a pooled analysis involving the 436 patients with metastatic colorectal cancer, accrued in the three aforementioned cohorts. The primary aim of such pooled analysis was to examine rest-activity rhythm as a circadian biomarker for the prediction of survival and progression-free outcomes in patients with metastatic colorectal cancer, with adequate statistical power. A secondary aim was to explore the relations between the rest-activity circadian rhythm and outcomes in the main patient subgroups. This analysis was made possible by the large sample size of the pooled dataset. Such results were required before a broad routine implementation of this biomarker in the framework of personalized patient-centered cancer chronotherapy.

MATERIALS AND METHODS

Patient cohorts and treatment regimens

The pooled analysis involved 436 patients with metastatic colorectal cancer who had been enrolled in any of the three cohorts that prospectively investigated the relations between rest-activity rhythm and patient outcomes from April 1994 to November 2006. The patients in these cohorts were subsets of clinical studies (Cohorts I and II) or had actimetry assessment as part of their routine chronotherapy management (Figure 1). Entry criteria in each of the three cohort studies included histological proof of colorectal cancer, measurable metastatic disease, WHO Performance Status less than 3, and signed written informed consent. Eligible patients had a physical examination, Computed Tomography (CT) scan of the thorax, abdomen and pelvis, a complete blood count, and serum chemistry including CEA and CA19-9. All the patients had rest-activity recorded with a wrist watch "Mini-motion Logger" for a minimum of 48 h up to 7 days. The patients subsequently received at least one course of conventional or circadian-based chemotherapy (chronotherapy) involving oxaliplatin (O), irinotecan (I) or both, eventually combined with 5-fluorouracil (F) and Leucovorin (L) (Levi et al., 1997; Gholam et al., 2006). ChronoFL consisted in four consecutive daily chronomodulated infusions of 5-fluorouracil (cumulative dose per course, 2800-3200 mg/m²) and leucovorin (1200 mg/m²) from 22:15 to 9:45, with peak delivery rate at 4:00 at night every 2 weeks (Giacchetti et al., 2000). ChronoFLO involved the addition of four daily chronomodulated oxaliplatin infusions from 10:15 to 21:45 with peak delivery rate at 16:00 (cumulative dose, 85-100 mg/m²) (Giacchetti et al., 2006). For chronoIFL, irinotecan (180 mg/m²) was infused from 02:00 to 08:00 with peak delivery at 05:00 on day 1, then followed by chronoFL (garufi et al.,2006). The chronoIFLO schedule combined day1 chronomodulated irinotecan (180mg/m²) with day2-5 chronoFLO (5-fluorouracil, 2800 mg/m², leucovorin, 1200 mg/m², oxaliplatin, 85 mg/m²) every 3 weeks (Gholam et al., 2006).

The characteristics of the patients in Cohorts I and II were previously reported (Mormont et al., 2000; Innominato et al., 2009a). The majority of the 142 patients in Cohort III (69%) had failed on prior chemotherapy for metastatic disease, while this was the case for none of the patients in Cohort II. Fifty-five percent and 33% of the patients in Cohort III had received prior oxaliplatin and irinotecan respectively, before rest-activity rhythm assessment. In contrast, these agents had been respectively administered to 1.8% and 5.4% of the patients in Cohort I (Mormont et al., 2000). The main chemotherapy protocols given after rest-activity assessment differed among the three cohorts. Patients in Cohort I received FL or FLO chronotherapy as first or second line chemotherapy (Giacchetti et al., 2000; Levi et al., 2007; Curé et al., 2002). Patients in Cohort II received first line chronoFLO or FOLFOX for metastatic disease (Innominato et al., 2009a; Giacchetti et al., 2006). Irinotecan-based chemotherapy was administered to 39.4% of the patients in Cohort III, and in none of the patients in either Cohort I or II.

Efficacy endpoints

Patients were followed for up to 10 years after actimetry recording. Response was assessed every third or fourth treatment course and defined according to WHO criteria (Jaffe, 2006). Independent radiology assessment was conducted for patients considered responders by the investigator. Patients were taken off the post actimetry treatment protocol as a result of progressive disease, lack of full recovery from severe toxicity, or complete surgical resection of metastases. Overall survival (OS) was defined as the time from first day of actimetry recording (day 0) to death or last time known alive. Patients who discontinued therapy for

reasons other than death or progression were censored at the drop out date. Progression-free survival (PFS) was also computed from day 0 to progression or death.

Rest-activity rhythm monitoring

To assess individual circadian rest-activity rhythm, a Mini-Motionlogger actigraph (Ambulatory Monitoring Inc., USA) was used. The actigraph looks similar to a watch and is worn on the non-dominant wrist. It contains a piezoelectric linear accelerometer to detect wrist movements and a memory chip for data storage. The time interval for the recording and count of activity level was 1 min. The actigraph was worn for at least 48 h continuously before the beginning of the first course of a new chemotherapy protocol.

Actimetry time series analyses

All actimetry time series were analyzed using a specific program (Action 4 version 1.10; Ambulatory Monitoring Inc., USA). The dichotomy index (I<O) was computed as the percent of time of activity in-bed, that is below the median level of activity out-of-bed (Mormont et al., 2000; Minors et al., 1996). Thus, the primary measure, I<O, essentially assessed the reduction in physical activity while in-bed (I) compared to that when out-of-bed (O). A normal dichotomy index is one approaching 100%, indicating restful sleep and regular daily activity. The I<O measure is based upon 24 h of actimetry readings. Visual inspection of each day's data indicated the approximate times of retiring and rising, based on sustained falls and rises in the activity counts, respectively. Activity counts during one hour before and one hour after each of these two times were ignored. This meant that a total of 20 h data per 24 h was considered, with a reasonable certainty that the data spans that were analysed indeed consisted of a section associated with attempted sleep (I, in bed) and another section associated with being awake (O,

out of bed). Daytime naps were included in the "awake" section. The median value of each of the two sections was then calculated, so that no assumption on data normalcy of distribution was required. I<O would be expected to approach 100% as a subject's dichotomy (between in-bed inactivity and out-of bed activity) increased. Values of I<O in healthy controls are rarely <98% (Minors et al., 1996). Lower values of I<O can be due to lower values of O and/or higher values of I. In practice, O (the median of all waking activity) rarely decreases sufficiently for it to exert a large influence on the index; it is usually a rise in activity counts during the time of attempted sleep that is the dominant factor in causing the I<O index to drop (Innominato et al., 2009a). Records examples and corresponding I<O values are shown in Figure 2. Neither the visual pattern of rest-activity nor the computed parameters were known by the clinician who prescribed the treatment protocol or took charge in patient management.

Statistical methods

Median and range were computed for I<O. The relations between patient characteristics and I<O upon study entry were tested using Kruskall-Wallis non-parametric tests for categorical variables and Pearson correlation for continuous variables. Median and interquartile range (IQR) were computed according to performance status, sex, number of metastastic sites and number of chemotherapy lines before actimetry measurements.

For illustrating the prognostic role of I<O on OS and PFS, patients were split in two groups according to I<O value either above or below I<O median value, and the corresponding Kaplan-Meier survival curves were plotted. Logrank tests were performed for testing equality of survival functions. Multivariate analyses were performed using Cox proportional hazard models to investigate the effect of I<O on OS and PFS, in each patient cohort and in the whole study population. Variables with $p<0.10$ according to univariate analysis were selected († in Table 1), and retained in the multivariate model using $p<0.05$ for the Wald test , using the forward stepwise selection procedure. In the pooled analysis, OS and PFS were analyzed

following stratification according to cohort, in order to account for differences in patient demographics, advancement of metastatic disease and treatment protocols after actimetry. These stratified Cox models allowed the underlying hazard function to vary across the 3 cohorts. For each model, I<O was used as a continuous variable, and the proportional hazards assumption was evaluated by plotting the martingale residuals. Forest plots were drawn to illustrate the effect of I<O within different patient subgroups and to visualize the adjusted hazard ratios estimated in the multivariate Cox models and representing the effect of I<O on OS and PFS in each patient cohort and in the whole study population. All analyses were performed using R 2.15.2 and PASW Statistics 20 software (SPSS, Chicago, IL, USA).

RESULTS

The circadian patterns in rest-activity differed largely among individual patients with similar characteristics. Such inter-patient differences appeared to be adequately recapitulated in the computation of I<O (Figure 2). In the pooled patient population, I<O varied according to performance status, ranging from a median value of 98.2 [IQR: 95.4 to 99.3] for PS=0, to 96.5 [93.1 to 99.0] for PS=1, and 91.5 [79.1 to 97.0]) for PS ≥2, with statistically significant differences in distribution (p from Kruskall-Wallis test <0.001). Median I<O was 97.1 [93.5 to 99.0] in males, and 98.0 [94.1 to 99.4] in females (p = 0.04). The I<O distribution also varied according to number of metastatic sites, the medians ranging from 99.4 [98.2 to 99.9] in the patients without any detected metastasis, to 97.9 [94.1 to 99.2] in those with one metastatic site, and 97.1 [92.7 to 99.0] in the patients with ≥2 metastatic sites (p = 0.03). Median I<O values were similar in chemotherapy-naïve patients (97.5 [93.5 to 99.2]), as well as in patients previously treated with one (97.7 [94.5 to 99.1]) or ≥2 chemotherapy protocols (97.2 [92.9 to 99.2]) (Kruskall-Wallis, p = 0.84).

Cohort III

The study enrolled 142 patients, who signed an informed consent form. Patients had poor prognosis disease as indicated by the administration of one to three conventional chemotherapy protocols to 69% of the patients, prior surgery for metastases among 41.5% of the patients, and prior administration of oxaliplatin and/or irinotecan for 55% and 33.1% of the patients respectively (Table 1). I<O ranged from 63.2 to 100%, with a median value of 98.3%. The patients subsequently received chronoFLO (47.9%), irinotecan-based combination chronotherapy (39.4%), or another schedule (12.7%).

Patients with I<O above its median value had a better overall survival than those with a lower I<O (p=0.006), with respective median survival of 23.3 months [95% Confidence.Interval, 13.6 to 33.0] versus 14.1 months [95% C.I., 9.1 to 19.2] (curves not shown). Similarly, median PFS was 7.4 months [95% C.I., 5.4 to 9.5] in the patients with I<O above 98.3% as compared to 5.1 months [95% C.I., 2.8 to 7.3] in those with lower I<O value (curves not shown). However, the PFS curves did not differ significantly according to median of I<O (p=0.068). Multivariate analysis revealed continuous I<O as an independent prognostic factor for overall survival, along with performance status, number of metastatic sites, number of prior chemotherapy protocols, and subsequent surgery for metastases (results not shown). The adjusted hazard ratio corresponding to a one percent increase of I<O was 0.953 [95% C.I., 0.924 to 0.984] (p=0.003).

POOLED PATIENT POPULATION

Patient characteristics

The updated patient data from the patients in Cohorts I and II were pooled with the patient data from Cohort III (Table 1). Overall, a total of 436 patients with metastatic colorectal cancer had adequate longitudinal assessment of their rest-activity rhythm for time series analysis (Figure 1). The pooled sample consisted of 37.4% women and 62.6% men from four countries. Fifty-one percent of the patients had two or more metastatic sites. Prior chemotherapy for metastatic disease had been administered to 45.4% of the patients. Following rest-activity rhythm assessment, chemotherapy was administered as a conventional modality for 71 patients (16.4%) (Giacchetti et al., 2000, Giacchetti et al., 2006) or as chronotherapy for 360 patients (82.6%). Chronotherapy included chronoFLO (266 patients) (Levi et al., 1997; Giacchetti et al., 2006), chronoFL (38 patients), or irinotecan-based chronotherapy (56 patients) (Mormont et al., 2000, Gholam et al., 2006; Garufi et al., 2006). Patients received a median number of 8 courses (range, 1 to 37) of the protocol that immediately followed actimetry recording. Surgery for metastases resection was performed in 22.4 % of the patients, following this new or a subsequent chemotherapy protocol.

I<O as a Prognostic Factor for Overall Survival

I<O ranged from 42.3% to 100%, with a median value of 97.5%. The median OS of the patients with I<O above the median value of 97.5% was 21.6 months [95% C.I., 17.8 to 25.5] as compared to 11.9 months [95% C.I., 10.4 to 13.3] in the patients with I<O equal to or less than 97.5% (Logrank, $p < 0.001$) (Figure 3A). The crude hazard ratios of I<O for overall survival were remarkably consistent across the nine main patient characteristics subgroups, including for patients with a PS of 0, 1 or 2 (Figure 4A). Results from multivariate analysis thus revealed that I<O was an independent prognostic factor for overall survival, along with performance status, number of metastatic sites, percentage of liver involvement, lung metastases, resection of primary tumor, and number of prior chemotherapy protocols (Table 2). The adjusted hazard ratio corresponding to a one percent increase of I<O was 0.954 [95%

C.I., 0.940 to 0.968] (p<0.001). Moreover the adjusted hazard ratios were remarkably similar in each of the three cohorts (Figure 4C).

I<O as a Prognostic Factor for Progression-Free Survival

The patients whose I<O value exceeded the median value of 97.5% had a median PFS of 9.3 months [95% C.I., 7.6 to 11.0], as compared to 5.8 months [4.5 to 7.1] for those with a I<O equal to or less than 97.5%. PFS curves differed significantly between both groups (Logrank, p<0.001) (Figure 3B). The crude hazard ratio values were also similar in each patient subgroup categorized according to the 9 main patient characteristics, including PS (Figure 4B). The variable selection procedure retained I<O and four other prognostic factors in the best fitting Cox model (Table 2). The hazard ratio for I<O was highly statistically significant, with HR=0.970 [0.956 to 0.984], and displayed similar values in each cohort, including the Cohort III model with I<O, following forcing into the model to obtain parameter estimate and confidence interval (Figure 4D).

DISCUSSION

The rest-activity circadian rhythm was measured using the actimetry parameter I<O, which is the percentage of physical activity during the night that is less than the median activity during the day (Minors et al., 1996). This parameter was selected for this pooled analysis, since it displayed consistent prognostic value for survival in two previous studies, while mean activity and other parameters did not (Mormont et al., 2000; Innominato et al., 2009a). Here, I<O first proved to be a robust predictor of overall survival over the subsequent ten years, independent of other risk factors, in a new cohort of 142 patients with metastatic colorectal cancer. This finding is consistent with those obtained in two previous cohorts composed of colorectal cancer patients with markedly different demographic characteristics, stage of disease

progression, and treatment protocols (Mormont et al., 2000; Innominato et al., 2009a). The combined prediction among the 436 patients analyzed together reveals that the circadian rest-activity rhythm measure robustly predicts overall survival. In particular, the patients with an I<O above 97.5%, indicating relatively little nighttime activity compared to median daytime activity, survived nearly twice as long as those with values equal to or below this value. The prediction was not simply an indication of circadian disruption when death was imminent, but rather a far longer-term indicator of outcome. A similar relation was statistically validated for I<O and PFS in the pooled patient population for the first time.

Rest-activity measured with I<O is thus a sensitive measure of circadian rhythms salient to cancer, that integrates poor sleep at night, fatigue and physical inactivity during the day, as well as anorexia, and pain. This measure is in fact associated with all four variables, which are under circadian control (Mormont et al., 2000, Innominato et al., Innominato et al., 2009a; Innominato et al., 2009b). These common cancer-related problems may interact and reinforce one another (Spiegel, 2008). This suggests the possibility that interventions designed to improve sleep and diet and encourage physical activity have the potential to improve quantity as well as quality of life (Butler et al., 2009 ; Palesh et al., 2007 ; Spiegel, 2011). Changes in of I<O value primarily reflect in-bed activity, the number of minutes with activity greater than the daytime median. Thus, changes in daytime out-of-bed activity would modestly affect the out-of-bed median, unless being substantial and sustained. As a result, the dichotomy index I<O is in part a measure of continuity and restfulness of sleep, and its prediction of cancer survival integrates sleep quality into the full circadian activity rhythm. Indeed, sleep is tightly regulated by the circadian timing system, with established links between the circadian rhythm in core body temperature and sleep onset, as well as sleep quality (Borbely & Achermann, 1992). Recent experimental data further show that increased activity during the usual activity span reinforces the robustness of the circadian signals arising from the pacemaker neurons in

the SCN (Meijer, 2011). In this context, poor I<O would indeed reflect both circadian disruption and poor sleep.

It appears in particular that colorectal cancer patients with I<O score of 97.5% or less are at substantially higher risk for disease progression and death. However, no statistical or clinical threshold was identified in our study. Rather, I<O was a continuous predictor of both OS and PFS, that fit the definition of a biomarker: "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Buyse et al., 2010, Wagner, 2008). The limitations of our study relate to the fact that the prognostic value of I<O was assessed in patients with metastatic colorectal cancer, at various stages of their metastatic disease, and on various chemotherapy protocols, though mostly including circadian-based chronotherapy. This study also does not rule out the hypothesis that a poor I<O could indicate a more aggressive type of cancer. Indeed, the patients with poorest I<O values displayed significantly higher levels of circulating TGFα, IL-6 or TNFα as compared to the patients with highest I<O. Indeed, these cytokines can alter circadian clocks in experimental models (reviewed in (Levi et al., 2010). Experimental circadian disruption through suprachiasmatic nuclei ablation or chronic jet lag however accelerated cancer growth in tumor-bearing mice 3 (reviewed in (Levi et al., 2010). In the current pooled analysis, the consistent effects of I<O in each category of established prognostic factors and its robust and independent effect on both survival outcomes support that circadian disruption indeed contributes to poorer survival jointly with six other variables. The consistent effects of I<O on OS and PFS in each cohort, as well as in each category of the nine main patient characteristics, further ruled out any significant impact of the different chemotherapy protocols that were delivered upon the measured effect. Conversely, the fact that 82.6% of the patients received chronotherapy

prevented any reliable comparative assessment of the predictive value of I<O for chronotherapy *versus* conventional treatment delivery.

Recent data show that chemotherapy delivery can also disrupt the rest-activity rhythm and result in "I<O" values equal to or below 97.5%. Interestingly, the overall survival of the patients with such circadian disruption was shorter than those who maintained an adequate rest-activity pattern while receiving chemotherapy (Innominato et al., 2012).

The feasibility of a routine assessment of this circadian biomarker at low cost is being greatly enhanced through the integration of rest-activity recording within telemedicine platforms aiming at improving quality of life of cancer patients at home (European InCASA project ICT-FP7) (Levi et al., 2012). The joint detection of circadian and sleep disruption through rest-activity monitoring and I<O quantification provides a robust indicator of the prognosis of patients with metastatic colorectal cancer which is independent of the usual clinical or biological prognostic measures. Interventions aiming at I<O enhancement, especially when less than 97.5%, through concomitant increase in lively out-of-bed activity and restful in-bed nighttime sleep, have the potential to improve overall survival, as well as progression-free survival, systemic symptoms, and quality of life of patients with advanced cancer.

Conflicts of interest

The authors declare no conflict of interest.

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Table 1: Main characteristics of the patients in the new Cohort, as well as in updated Cohorts I and II, and in the Pooled population.

Clinical and biological characteristics				
	Patient number (%)			
Characteristic	**Cohort I**	**Cohort II**	**Cohort III**	**Pooled population**
Age (years), median (range)	59 (21-78)	60 (22-75)	60 (21-83)	59 (21-83)
Sex				
Male	115 (68.5%)	71 (56.3%)	87 (61.3%)	273 (62.6%)
Female	53 (31.5%)	55 (43.7%)	55 (38.7%)	163 (37.4%)
PS (WHO) †				
0	102 (60.7%)	67 (53.2%)	84 (59.6%)	253 (58.0%)
1	52 (31.0%)	48 (38.1%)	44 (31.2%)	144 (33.0%)
2	14 (8.3%)	11 (8.7%)	13 (9.2%)	38 (8.7 %)
Unknown	0	0	1 (0.7%)	1 (0.2%)
Primary Tumor Site				
Colon	118 (70.2%)	95 (75.4%)	93 (65.5%)	306 (70.2%)
Rectum	50 (29.8%)	31 (24.6%)	49 (34.5%)	130 (29.8%)
Number of Metastatic Sites †				
None	0	1 (0.8%)	8 (5.6%)	9 (2.1%)
One	70 (41.7%)	56 (44.4%)	76 (53.5%)	202 (46.3%)
Two or more	98 (58.3%)	69 (54.8%)	58 (40.8%)	225 (51.6%)
Liver metastases †	137 (81.5%)	104 (82.5%)	103 (72.5%)	344 (78.9%)
Liver replacement by tumor †				
None	31 (18.5%)	22 (17.5%)	36 (27.5%)	89 (20.4%)
<25%	63 (37.5%)	59 (46.8%)	46 (35.1%)	168 (38.5%)
≥25%	74 (44.0%)	44 (34.9%)	49 (37.4%)	167 (38.3%)

Unknown	0	1 (0.8%)	11 (7.7%)	12 (2.8%)
Lung metastases †	71 (42.3%)	46 (36.5%)	53 (34.6%)	170 (39.0%)
BMI				
(18.5 ; 25)	88 (52.4%)	56 (44.4%)	72 (50.7%)	216 (49.5%)
<18.5 ; >25	77 (45.8%)	70 (55.6%)	69 (48.6%)	216 (49.5%)
Unknown	3 (1.8%)	0	1 (0.7%)	4 (1.0%)
Median plasma concentration (range) †				
Carcinoembryonic antigen, mg/L	44 (1-14780)	35 (0-20932)	57 (0-36845)	44 (0-36845)
CA19.9, IU/L	214 (1-78080)	82 (1-49038)	69 (1-23361)	127 (1-78080)
Treatment History Before and After Actimetry				
Before actimetry				
Prior surgery for				
Primary tumor †	160 (95.2%)	107 (94.9%)	134 (94.4%)	401 (84.9%)
Metastases †	53 (31.5%)	0	59 (41.5%)	112 (25.7%)
Prior chemotherapy				
Adjuvant situation	34 (20.2%)	21 (16.7%)	43 (30.7%)	98 (22.5%)
Metastatic disease †	100 (59.5%)	4 (3.2%)	98 (69.0%)	202 (46.3%)
Number of prior chemotherapy lines for metastatic disease †				
None	68 (40.5%)	126 (100%)	44 (31.0%)	238 (54.6%)
One	56 (33.3%)	0	35 (24.6%)	91 (20.9%)
Two or more	44 (26.2%)	0	63 (44.4%)	107 (24.5%)
Prior drugs given				
5-Fluorouracil	95 (56.5%)	0	93 (65.5%)	188 (43.1%)
Oxaliplatin	3 (1.8%)	0	78 (55.0%)	81 (18.6%)
Irinotecan	9 (5.4%)	0	47 (33.1%)	56 (12.8%)
Other	24 (14.3%)	0	12 (12.7%)	36 (8.2%)

After actimetry				
Surgery for metastases	53 (31.5%)	23 (18.3%)	20 (14.1%)	96 (22.4%)
Chemotherapy protocol				
ChronoFLO	137 (81.5%)	61 (48.4%)	68 (47.9%)	266 (61.0%)
ChronoFL	31 (18.5%)	0	7 (4.9%)	38 (8.7%)
Chrono Irinotecan ± FLO	0	0	56 (39.4%)	56 (12.8%)
FOLFOX2	0	65 (51.6%)	6 (4.3%)	71 (16.3%)
Other	0	0	5 (3.5%)	5 (1.1%)
Chronotherapy	168 (100%)	61 (48.4%)	131 (92.3%)	360 (82.6%)

† variables significant with p-value<0.1 in univariate Cox models for overall survival. Treatments after actimetry measures (baseline) were not candidate variables for the final Cox models.

Table 2: Results from Multivariate Analyses for Overall Survival and for Progression-free survival in the Pooled Population of 436 Patients with Metastatic Colorectal Cancer

Prognostic factor	Multivariate Cox Model Stratified by Cohort		
	HR	**(95 % CL)**	**p-value**
Overall Survival			
Rest-activity rhythm (I<O)	**0.954**	(0.940 – 0.968)	<0.001
Performance Status			<0.001
1 vs 0	**1.17**	(0.92 - 1.47)	0.19
2 vs 0	**3.99**	(2.71 – 6.00)	<0.001
Number of metastatic sites			<0.001
Two vs one	**4.49**	(1.34 – 15.02)	0.015
Three or more vs one	**8.92**	(2.57 – 30.94)	0.001
Metastatic liver replacement			0.001
1-24% vs none	**1.22**	(0.88 – 1.68)	0.23
≥25% vs none	**1.58**	(1.24 – 2.02)	<0.001
Lung metastases (yes vs no)	**0.67**	(0.51 – 0.89)	0.006
Prior chemotherapy protocols			<0.001
One vs none	**1.68**	(1.23 – 2.30)	0.001
Two or more vs none	**2.36**	(1.73 – 3.21)	<0.001
Primary tumor resected (yes vs no)	**0.52**	(0.35 – 0.77)	0.001
Progression-Free Survival			
Rest-activity rhythm (I<O)	**0.970**	(0.956 – 0.984)	<0.001

Performance Status			<0.001
1 vs 0	**1.09**	(0.88 – 1.36)	0.42
2 vs 0	**2.29**	(1.56 – 3.37)	<0.001
Number of metastatic sites			0.03
Two vs one	**1.52**	(0.73 – 3.17)	0.27
Three or more vs one	**1.93**	(0.92 – 4.04)	0.08
Prior chemotherapy protocols			<0.001
One vs none	**1.18**	(0.89 – 1.58)	0.26
Two or more vs none	**1.82**	(1.37 – 2.42)	<0.001
Primary tumor resected (yes vs no)	**0.50**	(0.34 – 0.73)	<0.001

LEGEND TO FIGURES

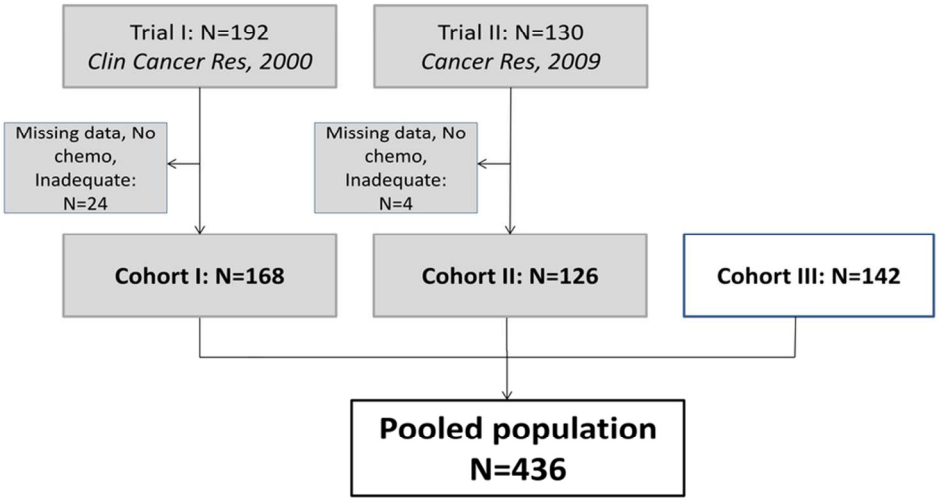
Figure 1: Diagram depicting the constitution of the three Cohorts and the Pooled Patient Population and the Studies they originate, whenever applicable.

Figure 2: Examples of baseline rest-activity records in two patients with metastatic colorectal cancer. Patient #1 displayed a robust circadian rest-activity pattern, with an I<O of 100%, while patient #2 had circadian disruption, with an I<O of 77.8%. Both patients #1 and #2, were 35 years old, had a WHO performance status of 0, and had received first line chemotherapy for liver-only metastases prior to entering the actimetry study. The survival of patient #1 was 37.5 months as compared to 16.6 months for patient #2.

Figure 3: Overall survival (A) and Progression-free survival (B) curves in pooled population of 436 patients according to baseline rest-activity rhythm as estimated with I<O. I<O was determined at time "0" shown on the abscissa. Survival curves are compared using Logrank test. Patients are categorized according to median I<O value of 97.5% for visualization purposes.

Figure 4: Forest plots of I<O effects on overall survival and progression-free survival according to (A, B) main patient characteristics, and (C, D) in each cohort and in the pooled analysis.

Figure 1



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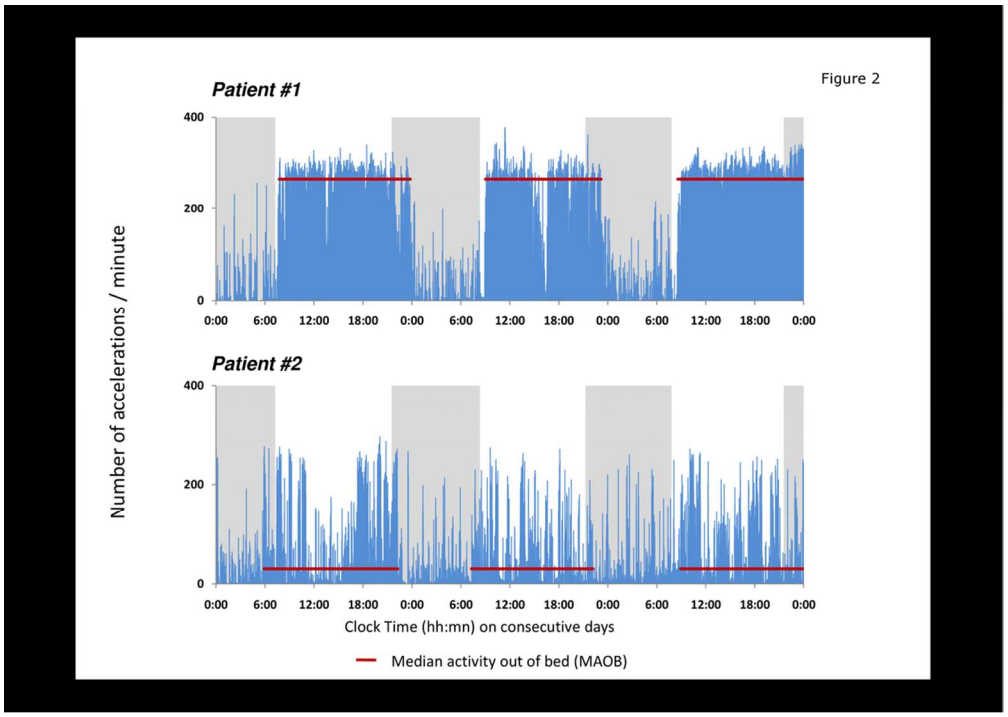

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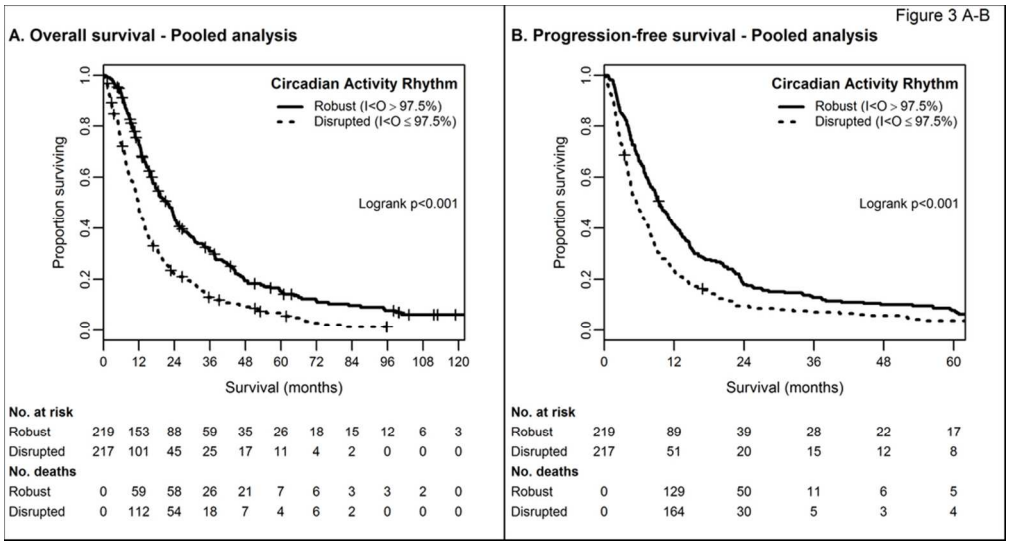

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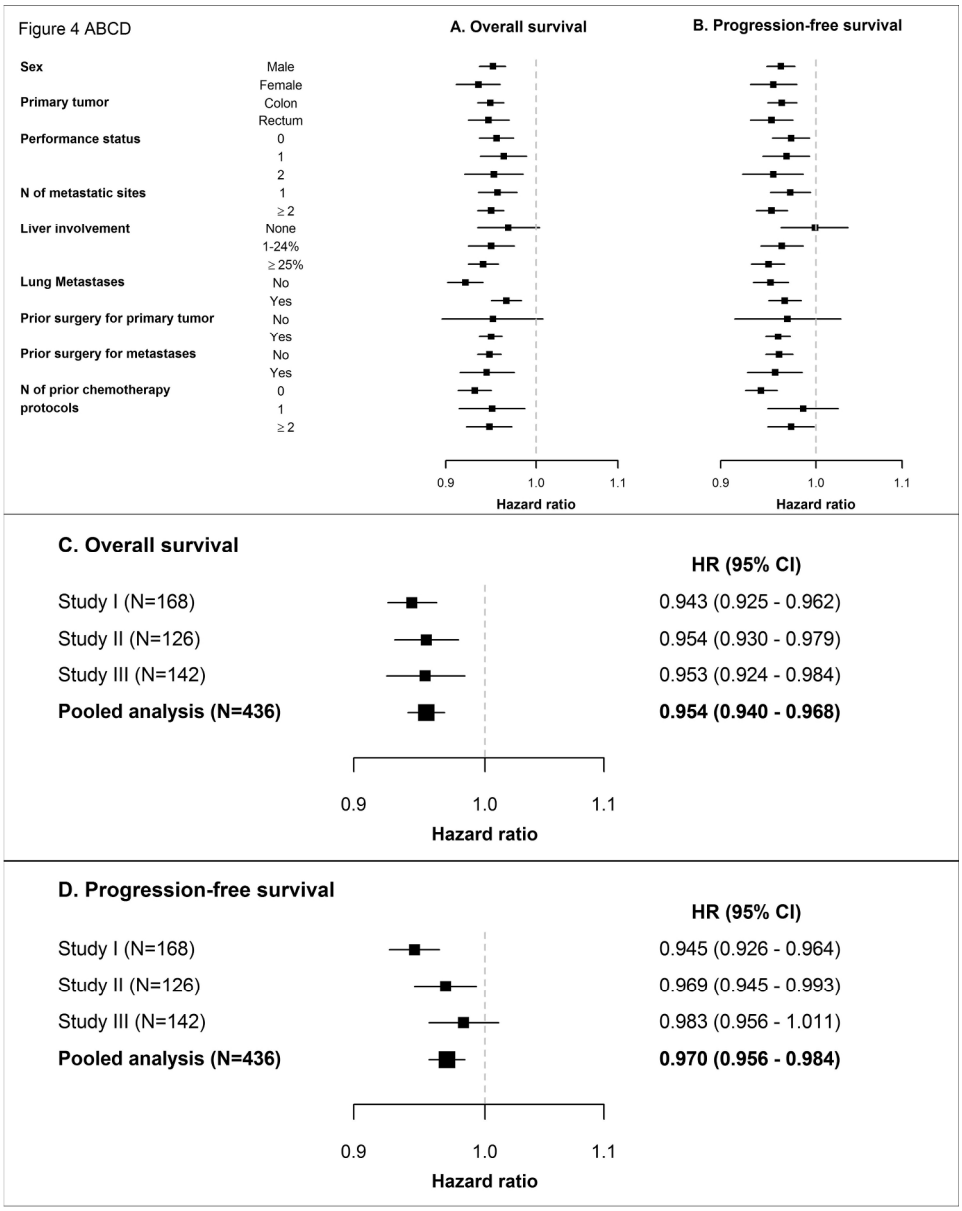

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